CLAIMS

1. A method for the quantitative detection of a nucleic acid (target) from a sample, which comprises the following steps:

a) extraction of the nucleic acid from the sample with another nucleic acid (calibrator) previously added to the sample itself, said calibrator having the same sequence of the target nucleic acid, with the exception of one region which in the target nucleic acid hybridizes with a probe labeled with a reporter and a quencher, that region of the calibrator, with respect to the corresponding region of the target nucleic acid, having the same nucleotide composition, but with a random sequence, and a similar Tm,

b) mixing the extracted target nucleic acid and calibrator with primers (forward and reverse) annealing to the corresponding regions on the calibrator and on the target nucleic acid, with probes bearing a reporter and a quencher and annealing to the target nucleic acid and to the corresponding randomized region on the calibrator, and with a nucleic acid polymerase with 5'-3' nuclease activity, in suitable conditions to carry out a polymerization reaction, and

c) determination of the signal associated with the reporters released due to the 5' polymerase nuclease activity.

2. A method for the quantitative detection of a nucleic acid (target) from a sample, which comprises the following steps:

a) extraction of the nucleic acid from the sample with another nucleic acid (calibrator) previously added to the sample itself, said calibrator having the same sequence of the target nucleic acid, with the exception of those regions which in the target nucleic acid hybridize with a probe labeled with a reporter and a quencher, and additionally hybridizing with two or more

primers, said regions having each other the same nucleotide composition, but with a random sequence, and a similar Tm,

b) mixing the extracted target nucleic acid and calibrator with primers (forward and reverse) annealing to the target nucleic acid and to the corresponding randomized regions on the calibrator, with probes bearing a reporter and a quencher and annealing to the target nucleic acid and to the corresponding randomized region on the calibrator, and with a nucleic acid polymerase with 5'-3' nuclease activity, in suitable conditions to carry out a polymerization reaction, and

c) determination of the signal associated with the reporters released due to the 5' polymerase nuclease activity.

3. Method according to claims 1-2, wherein the calibrator Tm is comprised in the ±4°C range of the target nucleic acid Tm.

4. Method according to claims 1-3, wherein the 5' end of the probes is 1 to 30 nucleotides from the 3' end of the forward primer.

5. Method according to claims 1-4, wherein the probes have the 3' end blocked in order to prevent the extension by the polymerase.

6. Method according to claims 1-5, wherein said nucleic acids, said probes and said primers are DNA sequences, and the nucleic acid polymerase is thermostable DNA polymerase with 5'-3' nuclease activity.

7. Method according to claims 1-6, wherein the probes have a Tm higher than that of the primers.

8. Method according to claim 7, wherein said probes include 18 to 30 nucleotides.

9. Method according to claims 1-8, wherein said probes include a quencher label able to reduce or to avoid the reporter label fluorescence when the probes are free in solution.

10. Method according to any of the preceding claims, wherein the target nucleic acid is genomic nucleic acid of the viruses HHV-6, HHV-7, HHV-8, HIV-1 and CAMV.

11. Method according to claim 10, wherein the virus is HHV-6, the forward primer has the sequence 5' CAAAGCCAAATTATCCAGAGCG 3', the reverse primer the sequence 5' CGCTAGGTTGAGGATGATCGA 3', the target nucleic acid probe the sequence 5' CACCAGACGTCACACCCGAAGGAAT 3', and the calibrator probe the sequence 5' TACGCAACGCCAACAGACCTAGCGA 3'.

12. Method according to claim 11, wherein the calibrator is additionally randomised in the regions annealing to primers having the sequences 5' CCGGAAACCGAACATTACTGAA 3' (forward) and 5' TTACGTGAGGATGATCGAGGC 3' (reverse).

13. Method according to claim 10, wherein the virus is HHV-7, the forward primer has the sequence 5' AGCGGTACCTGTAAAATCATCCA 3', the reverse primer the sequence 5' AACAGAAACGCCACCTCGAT 3', the target nucleic acid probe the sequence 5' ACCAGTGAGAACATCGCTCTAACTGGATCA 3', and the calibrator probe the sequence 5' TAAGCCCTGACCGCACGGGTATAATACTAA 3'.

14. Method according to claim 10, wherein the virus is HHV-8, the forward primer has the sequence 5' GTCCAGACGATATGTGCGC 3', the reverse primer the sequence 5' ACTCCAAAATATCGGCCGG 3', the target nucleic acid probe the sequence 5' CATTGGTGGTATATAGATCAAGTTCCGCCA 3', and the calibrator probe the sequence 5' ACTATTCCATGCGGAATTCGAGCATAGTTG 3'.

15. Method according to claim 10, wherein the virus is HIV-1, the

forward primer has the sequence 5' TACTGACGCTCTCGCACC 3', the reverse primer the sequence 5' TCTCGACGCAGGACTCG 3', the target nucleic acid probe the sequence 5' ATCTCTCTCCTTCTAGCCTCCGCTAGTCAA 3', and the calibrator probe the sequence 5'ACTCTCAGCGGCATTCTCCTCACTTCTACT 3'.

16. Method according to claim 10, wherein the virus is CAMV, the forward primer has the sequence 5' GTCTTGCGAAGGATAGTGGGA 3', the reverse primer the sequence 5' CACGTCTTCAAAGCAAGTGGA 3', the target nucleic acid probe the sequence 5' TGCGTCATCCCTTACGTCAGTGGAGAT3', and the calibrator probe the sequence 5'ATCGCTACATGCTAGGCATCTGTGTGC 3'.

17. Use of a calibrator, as defined in the preceding claims, in a method for the quantitative detection of a nucleic acid sample.

18. Kit for the quantitation of a nucleic acid from a sample, comprising one or more calibrators, a probe specific for each target nucleic acid and a probe specific for the calibrator, two or more primers and a thermostable nucleic acid polymerase with 5'-3' nuclease activity.